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**13. ABSTRACT (Maximum 200 words)**
Several soldiers from the Gulf War were wounded by depleted uranium (DU)-containing shrapnel. There is concern that DU may be more hazardous than other shrapnel because of its radioactivity and known toxicity to the kidney. Predictions of risk are necessary to guide the medical management of soldiers with DU-bearing wounds both now and in the future. We are determining the carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive foreign-body fragments and assessing the potential for renal toxicity of DU fragments by correlating urine and kidney concentrations of U with time after implantation. DU fragments of differing sizes and shapes are being implanted in the soft tissue of rodents to compare with results from animals implanted with inert metals. In this way a toxicity ratio will be determined that can be used to predict the expected response in humans from the known response of humans to relatively inert shrapnel. To date, a pilot study has been completed to determine important experimental design parameters for studying the foreign-body response using this test system in animals. Parameters defined include fragment *in vitro* and *in vivo* solubility, optimal fragment size and shape for implantation, changes in the surface characteristics of fragments that could be important in carcinogenesis, and determination that fragments of DU alloyed with titanium DU(Ti) would be more desirable than nonalloyed DU based on particle solubility. A long-term carcinogenesis study of DU fragments implanted in the muscles of rats has been initiated.

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Stefan F. Hahn 2/4/97

PI – Signature Date

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# I. INTRODUCTION

## A. Purpose and Scope of Work

The purpose of this work is to determine the relative carcinogenicity of depleted uranium (DU) fragments embedded in soft tissues and the renal toxicity from the chronic exposure to systemic uranium (U). These determinations will be made from the study of rats exposed to embedded DU. Results of these studies will be used to estimate the carcinogenicity and renal toxicity of DU embedded in humans.

Two hypotheses are being tested:

1) ***Depleted U-0.75% Ti alloy [DU(Ti)] is more carcinogenic in muscle tissues than tantalum (Ta) metal.*** We theorize that the chronic low-level irradiation of tissues surrounding embedded DU(Ti) fragments will increase the carcinogenic potency of the metal fragments. The objective of testing this hypothesis is to determine the relative risk of radioactive fragments relative to nonradioactive fragments so that informed judgements can be made about the clinical management of veterans with DU(Ti) fragments embedded in their soft tissues.

The specific aim of this study is to determine experimentally the relative carcinogenicity of radioactive DU(Ti) and a nonradioactive inert metal, Ta. This relative carcinogenicity in rats will be used in a ratio, so that the carcinogenicity of DU(Ti) in humans can be estimated using the following relationship:

$$\{\textbf{DU(Ti) toxicity/inert metal toxicity}\}_{\text{rat}} \_ \{\textbf{DU(Ti) toxicity/inert metal toxicity}\}_{\text{human}}$$

This approach is similar to the toxicity ratio method previously described to compare the risk of radiation-induced bone cancer in dogs and humans[1] and mice and humans.[2] The information for inert metal toxicity in humans is foreign-body carcinogenesis data related to metals used for implants, stainless steel, shrapnel, etc. [3–5] Ta is physiologically inert and has been used in metal implants in animals and humans for many years.[6] Thorotrast® is the positive control for radioactive material in rats. The responses in rats will be related to carcinogenic responses in humans exposed to Thorotrast® deposited in subcutaneous locations.[7]

It is well known that rodents are much more sensitive to foreign-body carcinogenesis than humans.[8] Thus, the direct test of carcinogenicity is rigorous and should not yield a false-negative result. On the other hand, a positive result cannot be extrapolated directly to the human situation, only the relative effect. To emphasize this point, a group of Thorotrast®-implanted rats serve as a positive control group whose results can be compared directly to the sarcoma incidence of human patients with

perivascular deposits of Thorotrast®.[7] Rats with surgical manipulations similar to those used to insert the implants are sham controls.

The incidence of subcutaneous tumors will be compared among dose groups by using a Cox proportional hazards (CPH) model. These types of models take into account not only the total incidence of tumors but also the times at which the tumors occur in order to obtain more power to test for statistically significant differences and to provide additional insight into the process of carcinogenesis. The relative risks estimated from the model for comparing DU to an inert metal in the rat are toxicity ratios of the two materials that will then be applied to humans using the observed foreign-body toxicity of inert metals such as Ta. Because data are limited on foreign-body carcinogenesis in humans (e.g., Ref. 3), we can only define an upper limit for toxicity. Data from rats injected with Thorotrast® will also be analyzed with a CPH model to understand the role of radiation dose and provide another comparison with humans.

2) ***Urinary concentrations of U are directly correlated with the renal concentrations of U and will reach a steady state after intramuscular implantation of DU(Ti) fragments.*** The objective of testing this hypothesis is to determine if the renal concentration of U reaches a steady state, and, if a steady state is achieved, what is the renal concentration and the corresponding urinary concentration of U. These data will enable informed judgements to be made about the potential renal damage in veterans who are excreting U in their urine from DU(Ti) fragments embedded in their tissues.

The specific aims of this study are to determine 1) the time course to achieve a steady-state renal U concentration from an implanted DU source and 2) whether toxicity is present at the steady-state concentration. In response to earlier reviews, this portion of the project is restricted to work that will obtain as much information as possible about the renal toxicity of U in the animals implanted with DU fragments and held for long periods. Accordingly, the scope of these studies is limited.

B. Nature of the Problem

Several soldiers who participated in Operation Desert Storm were wounded while in Bradley armored personnel carriers hit directly by armor-penetrating projectiles containing an alloy of DU and 0.75% titanium [DU(Ti)]. Elevated concentrations of U were detected in the urine of two such subjects, and shrapnel fragments were visualized radiographically in at least one subject at least 1 y after exposure documenting that detectable U may be present in exposed individuals for prolonged times. Subsequently, about 22 soldiers potentially bearing DU(Ti) have been identified and are being studied to

determine U excretion and possible biological effects.[9,10] These studies indicate that humans are being chronically exposed to U in both insoluble forms as DU(Ti) fragments and soluble forms as dissolving U.

Quantitation of the long-term health risk from exposure of humans to U, particularly in the form of embedded fragments, is complex and involves both chemical and radiological components, as well as possible foreign-body effects. Because of the unique features of these exposures, it is not currently possible to confidently predict the carcinogenic risks to these soldiers from their U-bearing wounds. Such predictions are necessary, however, to guide the medical management of soldiers with U-bearing wounds both now and in the future. There are no known studies of the long-term effects of U metal implanted in tissues.[10]

To assess the carcinogenic risks associated with long-term exposure to DU(Ti)-containing shrapnel in wounds, we are conducting studies in rats to determine the carcinogenicity of radioactive DU(Ti) fragments in tissues relative to nonradioactive metallic foreign-body fragments of Ta. Once a relative carcinogenicity factor is determined in rodent model systems, it can be used to compare the carcinogenicity of DU(Ti) with the known carcinogenicity of metal fragments in humans.

To assess more confidently the carcinogenic risks associated with long-term exposure to DU-containing shrapnel in wounds, the following work is being undertaken:

- The carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive foreign-body fragments is being determined.
- Urine and kidney concentrations of U are being correlated with time after implantation of DU fragments.

C. Background of Previous Work

**What do we know about the carcinogenicity of embedded fragments, either radioactive or nonradioactive?**

*Human data:* There are very few situations involving people where cancer has resulted from radioactive fragments embedded in the tissues. Scar formation with central liquefactive necrosis has been reported in association with intradermal plutonium metal deposits in plutonium machinists.[11] The late effects of these deposits are not known, however, because excision of the deposited material was the treatment of choice in these accident cases.

Thorotrast®, an X-ray contrast medium containing radioactive $^{232}Th$ as colloidal thorium dioxide, is known to cause tumors in the soft tissues of humans.[12] Inadvertent perivascular injection of

this material results in the local formation of fibrous tissue (thorotrast granulomas) in as many as 10% of the patients. In one study with incidence data, one metastasizing soft tissue sarcoma developed 30 y after injection in 142 patients with thorotrast granulomas.[7]

The incidence of cancer associated with nonradioactive fragments or foreign bodies in the tissues is important information because of the lack of data on radioactive fragments. There are no epidemiologic studies and only a few case reports of foreign-body carcinogenesis in humans, however, which indicate that the overall incidence must be low. Case reports and a literature review[13,14] showed only 40 cases of sarcoma associated with metallic foreign bodies, such as shrapnel, or metallic implants, such as protheses. Another review notes that the risk of implanted medical devices must be very low because only about two foreign-body neoplasms are reported per year.[4] A risk assessment of the cancers associated with implanted protheses concluded that the risk must be small because the incidence of these cancers was low in the face of an increasing usage of such protheses.[3] The assessment also included failed attempts to isolate "precancerous" cells from tissues around the implants using cellular culture techniques, similar to those used in studies of foreign-body carcinogenesis in rodents.

*Animal studies:* In contrast to humans, foreign-body tumors have been frequently induced in rats[15] and mice.[16] The development of sarcomas near the site of subcutaneous implantation of metal or polymer films in rodents is a well-described experimental result.[16,17] Foreign-body carcinogenesis appears dependent on a specific sequence of four events: 1) cellular infiltration and proliferation during the acute reaction, 2) fibrosis of the tissue capsule surrounding the foreign body, 3) quiescence of the tissue reaction, and 4) clonal expansion of preneoplastic cells with direct contact on the foreign body.

The physical shape and characteristics of the implant, not the chemical reactivity, appear to be essential for tumor induction. Smooth-surfaced films, with a relatively large area, induce tumors with a high efficiency, while the same films minced into small fragments, but with the same surface area, have significantly reduced tumorigenicity.[16] The presence of the foreign body is essential only during the first months of the latent period. Recent work has shown that implantation of foreign bodies after injection of ethyl nitrosourea or after whole-body gamma irradiation also leads to increased sarcoma development.[18] Thus, foreign bodies with less than the critical surface area for carcinogenesis may act as promoters of subcutaneous carcinogenesis initiated by other agents, including radiation.

Thorotrast® causes tumors in laboratory rodents, including tumors of the soft tissues (Bauer, 1948 as cited in Ref. 7). For example, 29 of 54 Chinese hamsters injected intravenously with a

relatively high dose of Thorotrast® (> 0.4 Bq/g) developed fibrosarcomas from perivascular leakage of some injections.[19] Plutonium fragments have been injected into the footpads of dogs to simulate the plutonium-contaminated wounds of plutonium machinists.[20] The plutonium was translocated to the local lymph nodes where it caused fibrosis but no tumors.

The available literature provides little guidance for directly evaluating the carcinogenicity of DU fragments in soft tissues. Based on cancer incidence data from people with nonradioactive foreign bodies, DU fragments do not appear to present a significant risk for causing cancer. However, there are indications from the foreign-body carcinogenesis studies in rodents and from the human experience with Thorotrast® that radioactive foreign bodies may be more carcinogenic than nonradioactive foreign bodies.

**What do we know about the renal toxicity of U?**

*Human data:* Uranium protection standards for humans are based on the chemical nephrotoxicity of U.[21–23] The basis for these standards was extensively reviewed in 1973.[24,25] A critical level of U, at which renal damage could be expected, was defined as a peak concentration of 3 μg/g kidney, a judgement based on the best data available at the time. Subsequent experience indicates that adherence to the present limits for exposure to U, which are based on this critical level, appears to provide adequate protection against U nephrotoxicity.[25] For example, a study of 31 workers exposed acutely to an accidental release of U hexaflouride resulted in renal U concentrations of 0.05 to 2.5 μg U/g kidney, but no worker had evidence of renal damage.[26] However, another study of renal function in healthy U mill workers has shown a slight increase in urinary amino acids and proteins, indicative of reduced proximal renal tubular resorption.[27] These changes are consistent with nephrotoxicity and are found in workers with the highest potential for chronic exposure to soluble U. These findings raise concern for the possible renal toxicity of chronic low-level exposure to U.

*Animal studies:* The renal toxicity of U has been extensively studied in animals, particularly in rats[28–31] and dogs[31,32]. Dogs appear more susceptible to nephrotoxicity than humans and less susceptible than rats.[30] A single injection of uranyl nitrate is a classic method for producing renal damage.[33] Necrosis of the terminal segments of the proximal renal tubule is characteristic for all species. At 1 mo after a single exposure in rats, there is a thinning of the proximal tubular epithelium, the result of regeneration of the necrotic epithelium.[28] Studies of rats repeatedly injected with U have shown that renal lesions are first seen when the renal U burden is between 0.7 and 1.4 μg/g and are most severe when the burdens are 3.4-5.6 μg/g. Repair is rapid; within 35 d the epithelium is normal.[30] Studies of

rats with constant 14-d perfusion of U (with osmotic pumps) have shown that renal toxicity is detected at renal U burdens of 1-2 μg/g.[34] These results and others in humans[27] have suggested that existing data on U nephrotoxicity should be reevaluated, particularly for chronic exposures.[35,36]

## II. BODY OF REPORT

### A. Pilot study

#### 1. Experimental Methods

It was necessary to clarify a number of critical variables before planning a relative carcinogenesis study in rodents. The purpose of this pilot study (Table 1) was to address three of these critical variables by determining the following: 1) the *in vivo* solubility of DU during the first 60 d after its implantation in rats and mice, 2) any changes in the surface characteristics of the DU foil after implantation, and 3) histological responses of rats and mice to the implanted DU during this time. Two types of foils containing DU were used. One contained only DU, while the other was the DU alloyed with 0.75% Ti which is identified as DU(Ti). The DU foils (20 mm × 15 mm × 1.6 mm) and DU(Ti) foils (20 mm × 15 mm × 1.5 mm) were obtained from Manufacturing Sciences Corporation (Oak Ridge, TN). Ta foils (Goodfellow Corp., Berwyn, PA) of similar size (22 mm × 15 mm × 1.1 mm) were used as the control implant. The composition of the DU(Ti) pellets is the same as that used in a study at the Armed Forces Radiobiology Research Institute (AFFRI) on the dissolution of DU(Ti) pellets in rats[37] and has been described in detail.[38]

Table 1

Experimental Design for the Study of Dissolution and Excretion of Uranium and Early Biological Effects of Subcutaneously Implanted DU, DU(Ti), or Ta Metal Foils in Male Rats and Mice

| | Foil Type and Number of Animals Sacrificed at 30 d | | | Foil Type and Number of Animals Sacrificed at 60 d[a] | | | |
|---|---|---|---|---|---|---|---|
| Rodent | DU | DU(Ti) | Ta | DU | DU(Ti) | Ta | Total |
| F344 Rats[b] | 5 | 5 | 4 | 5 | 5 | 4 | 28 |
| CBA/J Mice[b] | 5 | 5 | 4 | 5 | 5 | 4 | 28 |
| Total | 10 | 10 | 8 | 10 | 10 | 8 | 56 |

[a]Twenty-four hour urine samples were collected from three rats and three mice on days -2, -1, 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, and 60 before and after DU and DU(Ti) foils were implanted and on days -2, 7, 14, 28, and 35 before and after Ta foils were implanted.
[B]deaths of animals prior to the scheduled sacrifice time are discussed in the text.

The experimental design for the *in vivo* portion of this study is summarized in Table 1. Twenty-eight 12-wk-old male F344 rats (Charles River Laboratories, Wilmington, MA) and 28 12-wk-old male CBA/J mice (Harlan Sprague-Dawley) were used. Animals were housed in filter-topped polycarbonate cages on hardwood chip bedding or in metabolism cages. Animal rooms were maintained at 20 to 22 C° with a 40 to 60% relative humidity on a uniform 12-h light cycle. Food (Lab-Blox, Allied Mills, Chicago, IL) and water were available *ad libitum*.

The metal foils were weighed (mean foil weights ± SD; DU 8.4 ± 0.2 g, DU(Ti) 7.4 ± 0.3 g, and Ta 5.6 ± 0.1 g) and surgically inserted in the subcutis of the upper part of the back region of rats and mice while under halothane anesthesia. A sterile field was prepared over the anterior dorsum, and a surgical incision about 2.5 cm long in the skin was made to place sterile foils into the subcutis. The surgical site was closed with surgical wound clips, which were removed about 7 d after surgery. Twenty-four-hour urine samples were collected from rats and mice housed in metabolism cages (Table 1).

Rats and mice were sacrificed (Table 1) using a sufficient amount of pentobarbital given by intraperitoneal injection, and necropsied. The tissue capsules around the metal foils were removed, along with the heart-lung block, liver, kidney, femur, urinary bladder, epididymis, testes, and lesions, and were fixed with 10% neutral buffered formalin. Tissues were sectioned at 5 μm, and sections were stained with hematoxylin and eosin. The left kidney, tissue capsule, and lesions were examined histopathologically.

2. Results

The daily U excretion data for individual rats is summarized in Fig. 1. The urinary excretion of U appeared to increase throughout the study in rats with implanted DU foils. In contrast, the excretion of U by rats with implanted DU(Ti) increased rapidly until about 15 to 20 d, after which the daily excretion was relatively constant. The concentrations of U in the kidneys of rats with DU and DU(Ti) foil implants followed a similar pattern, i.e., at 30 and 60 d greater concentrations of U were in the kidneys of rats with the DU(Ti) implants than in the rats with the DU foil implants (Fig. 2).

Similar urinary excretion patterns were seen in mice, except that the rate of excretion of U in the mice implanted with DU(Ti) was not constant after 15 to 20 d as in the rats, but continued to increase (Fig. 3). The dissolution of U in the mice with the implanted DU(Ti) resulted in the accumulation of toxic levels of U in the kidneys (Fig. 4) resulting in the death of all but one mouse within 30 d, compared with the death of only one mouse with implanted DU. In contrast, only one rat with a DU(Ti) implant died, but not until day 33. The DU(Ti) foils were more soluble in both rodents

than the DU foils in either species. The translocation of U to the kidney and skeleton also indicated that the DU(Ti) foils were more soluble that the DU foils.

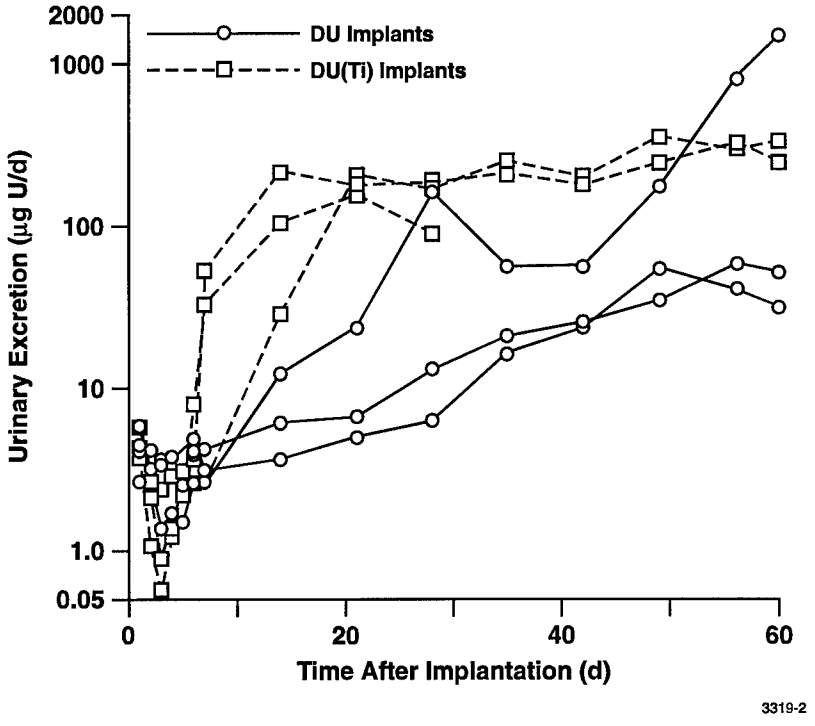



**Fig. 1.** Urinary excretion of U in µg $day^{-1}$ in individual rats with DU or DU(Ti) implants.

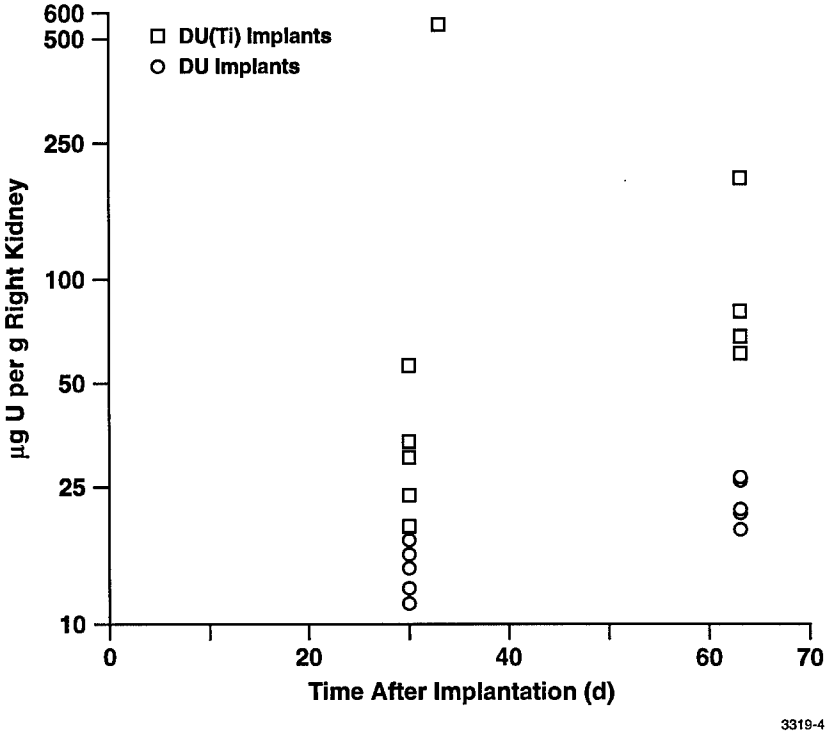



**Fig. 2.** Renal concentrations of U in rats with DU or DU(Ti) implants.

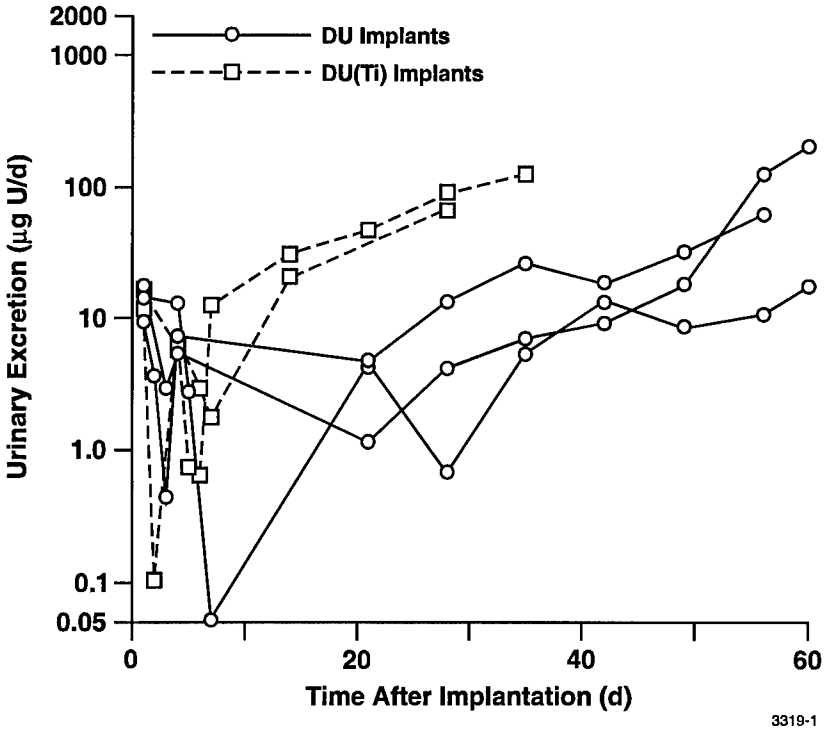



**Fig. 3.** Urinary excretion of U in mg day$^{-1}$ in individual mice with DU or DU(Ti) implants.

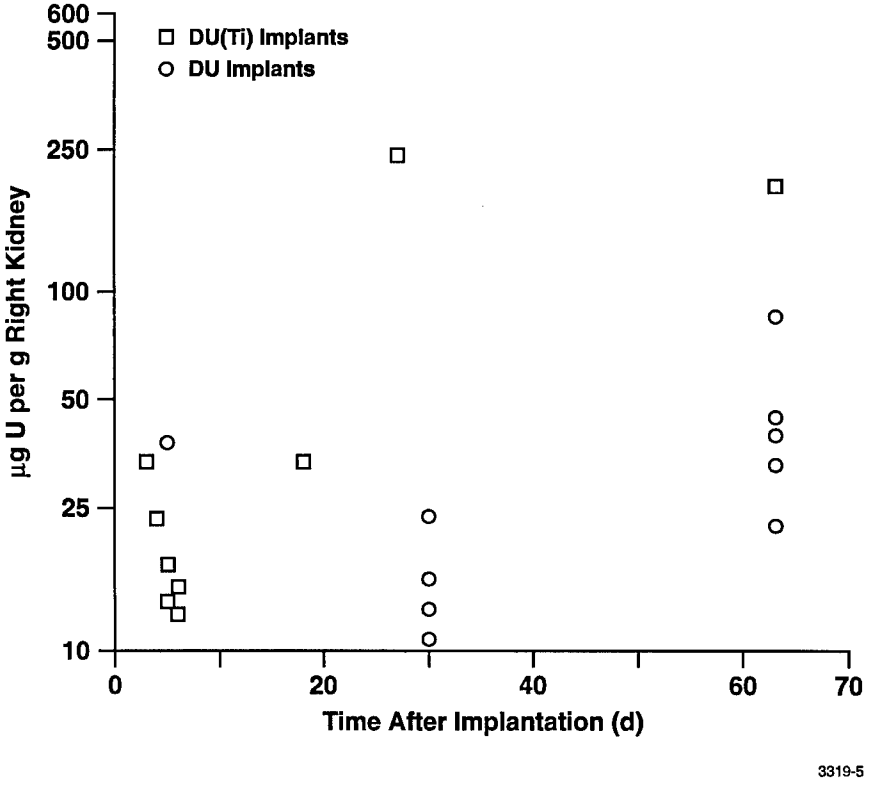



**Fig. 4.** Renal concentrations of U in mice with DU or DU(Ti) implants.

Results of in vitro solubility tests of 30 d with the DU and DU(Ti) foils (Fig. 5) were consistent with data from the implanted foils. In both solvent systems tested, water adjusted to pH 5.0 with HCl and serum simulant ultrafiltrate, the DU foils were more soluble than the DU(Ti) foils.

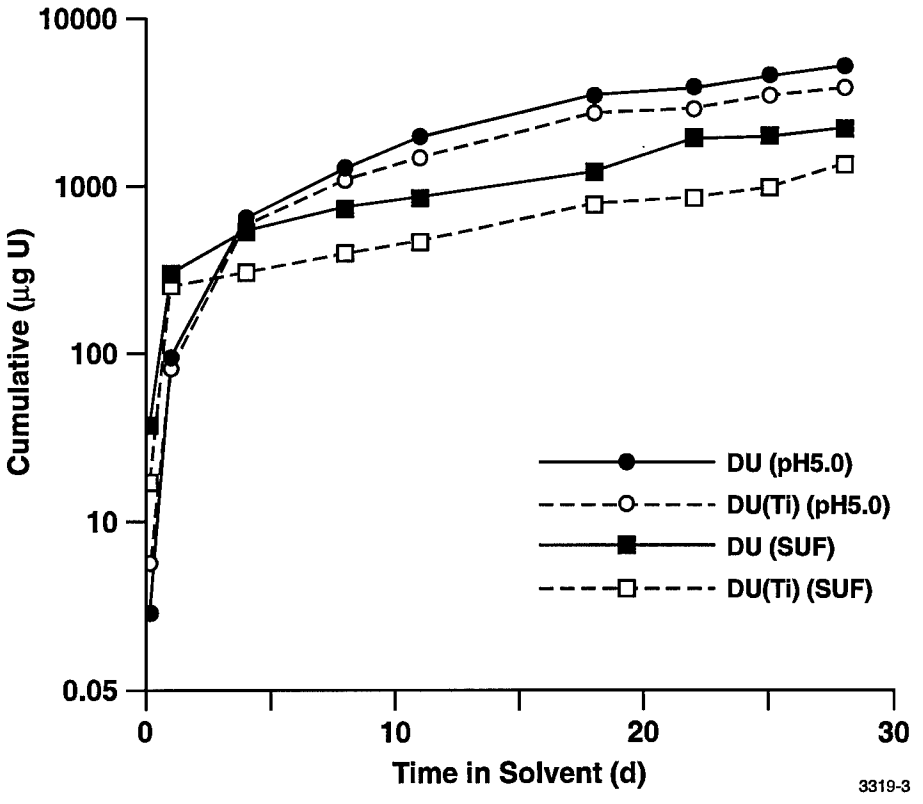



**Fig. 5.** *In vitro* dissolution of DU and DU(Ti) foils in two different solvents: Water acidified to pH 5.0 with HCl and serum simulant ultrafiltrate (SUF).

Thirty days after implantation in the subcutis, the physical appearances of both the DU and the DU(Ti) foils were markedly altered. The surfaces were roughened and friable, with small black particles or flakes coming off the foils. The flaked particles blackened the lining of the connective tissue capsule surrounding the foils. Scanning electron microscopy graphically showed the roughening of the surface of both the DU and DU(Ti) foils (Fig. 6). Angular particles were present on the surface of the DU foils and smaller, granular particles were present on the surface of the DU(Ti) foils. The significance of this difference in appearance is not known, but is further evidence that the two types of foils do not act the same in tissues. This appearance was slightly accentuated at the 60-d sacrifices. Similar changes in the surface of the DU and DU(Ti) foils were noted in those tested in the *in vitro* solubility system.

**a**) DU control (1000×)

**b**) DU(Ti) controls (1000×)

**c**) DU after 30 d of implantation (1000×)

**d**) DU(Ti) after 30 d of implantation (1000×)

**Fig. 6.** Scanning electronmicrographs of foil surfaces.

Histopathological examination of the tissue capsules surrounding the implants showed marked differences between the DU and DU(Ti) foils and the Ta foils. Around the Ta foils, there was a thin connective tissue capsule in some animals, with a scant infiltration of chronic inflammatory cells. The DU and DU(Ti) foils were surrounded by a moderately thick connective tissue capsule with moderate infiltration of chronic inflammatory cells (Fig. 7). Black particles were found embedded in the capsules.

Histopathological examinations of the kidneys showed a chronic tubular necrosis, which was severe enough to cause death before the 60-d sacrifice. The severity of the lesions was generally correlated with the concentration of U in the kidney.

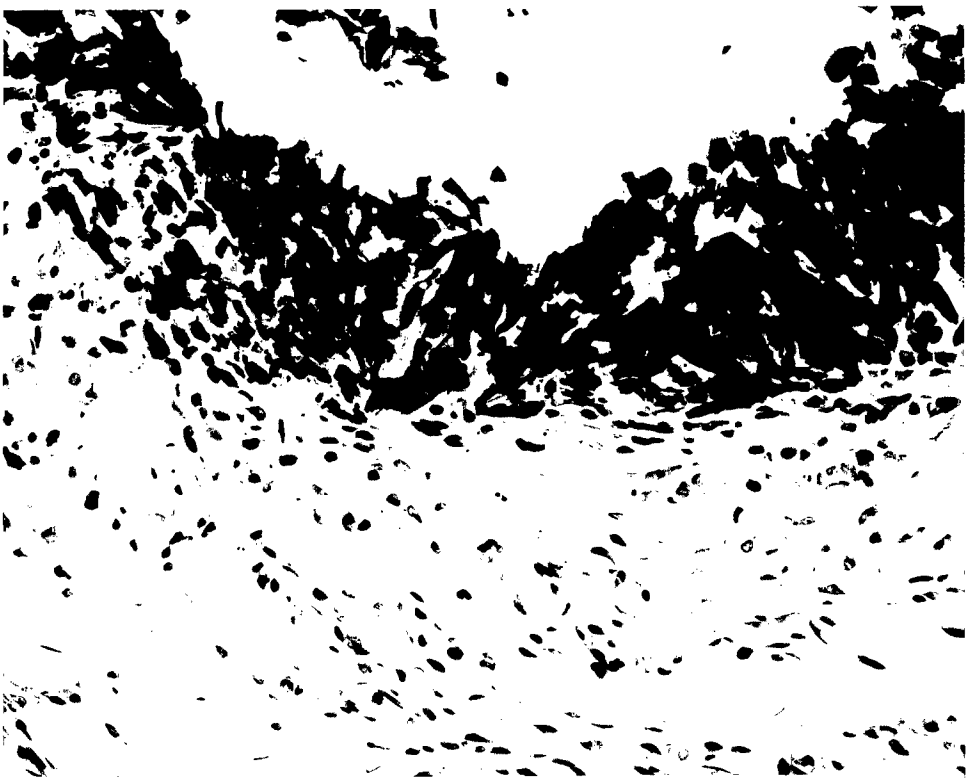
3. Conclusions

The pilot study indicates that DU and DU(Ti) foils dissolve more rapidly in mice and rats than expected and that DU(Ti) dissolves more rapidly than DU. In addition, both types of DU foils break down in the subcutis, becoming roughened and causing a moderate inflammatory cell infiltration in the surrounding tissues. DU(Ti) foils caused more inflammation and more renal damage. Both of these effects may relate to the greater solubility of DU(Ti).

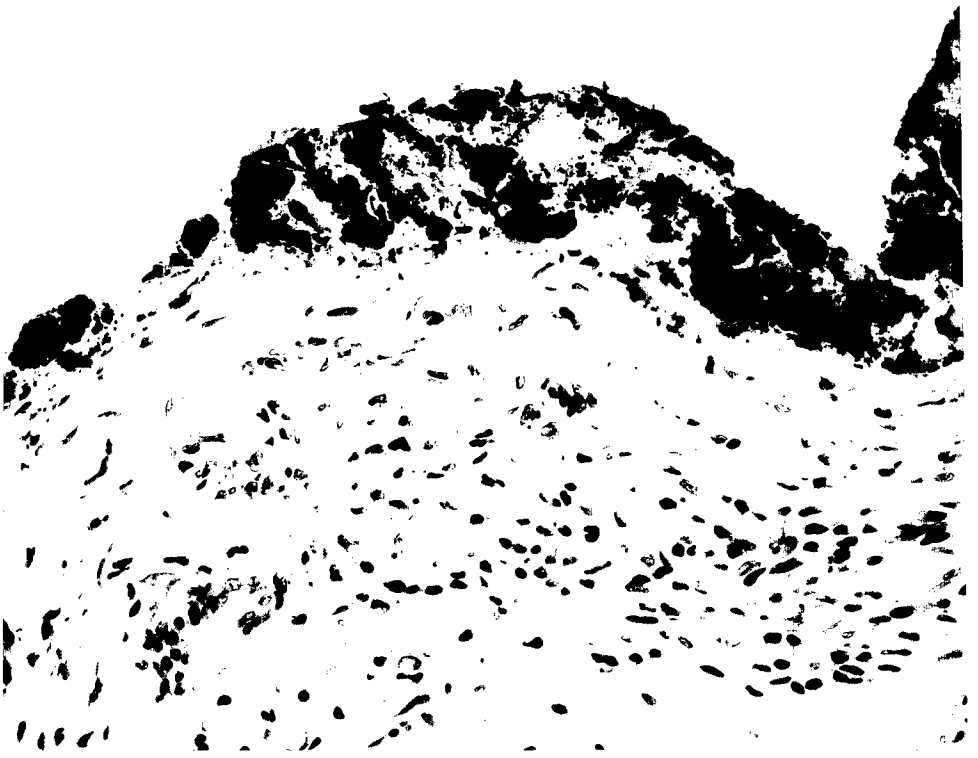
With the results from both species and following the flow chart outlined in Table 2, it is evident that the subcutaneous foreign-body carcinogenesis system described by Brand *et al.*[16] cannot be applied to a study of the carcinogenesis of implanted foils containing DU. Key elements in the Brand system are a smooth surface and a relative lack of inflammation. Therefore, results of the pilot study indicate that the bioassay carcinogenesis study in rats should be conducted using intramuscular implants of DU(Ti) in the form of small pellets and fragments, rather than in the form of foils.

B. Carcinogenesis Study

The pilot study (Table 1) provided information essential for the decision-making process (Table 2) to be followed developing a long-term carcinogenesis study with intramuscularly implanted DU(Ti) pellets and fragments in male Wistar rats (Table 3). The DU(Ti) pellets and fragments to be used in this study approximate the size of some of the DU(Ti) fragments imbedded in soldiers wounded in the Gulf War. Cylindrical DU(Ti) pellets (2.0 mm long × 1.0 mm in diameter) have been obtained from Manufacturing Sciences Corporation, Oak Ridge, TN. Two sizes of DU(Ti) fragments (2.5 mm × 2.5 mm × 1.5 mm and 5.0 mm × 5.0 mm × 1.5 mm) will be cut from DU(Ti) foils similar to those used in our pilot study.



**a**) capsule surrounding DU foil 60 d after implantation



**b**) capsule surrounding DU(Ti) foil 60 d after implantation.

**Fig. 7.** Photomicrographs of tissue responses.

Table 2

Flow of Studies and Decision Points

| Pilot Study of DU or DU(Ti) Foils in Subcutis of Rats or Mice | | | |
|---|---|---|---|
| Effects | | Solubility | |
| • Foils break down<br>• Foils initiate intense, protracted inflammation | | • Foils soluble<br>• U detected in kidney | |
| Yes | No | Yes | No |
| Initiate bioassay carcinogenesis study | Initiate Initiation/promotion carcinogenesis study | Initiate U analysis in carcinogenesis study | Delete U analysis in carcinogenesis study |

Table 3

Experimental Design for a Two-Year Carcinogenesis Study of DU(Ti) Pellets and Fragments Intramuscularly Implanted in Male Wistar Rats

| Type of Implant | Size (mm) | Number of Implants | Total Number of Rats[a] |
|---|---|---|---|
| DU(Ti) pellets | 2.0 × 1.0 dia. | 4 | 50 |
| DU(Ti) fragments | 2.5 × 2.5 × 1.5 | 4 | 50 |
| DU(Ti) fragments | 5.0 × 5.0 × 1.5 | 4 | 50[b,c] |
| DU(Ti) fragments | 2.5 × 2.5 × 1.5 | 4 | 36[b,c] |
| Thorotrast® injection | 0.025 ml | 2 | 50 |
| Ta fragments | 5.0 × 5.0 × 1.1 | 4 | 50 |
| Sham implant surgery | NA | 0 | 8[c] |
| Sham implant surgery | NA | 0 | 50 |
| Total number of rats | – | – | 344[d] |

[a]Any rats that die within 48 h of the implantation surgery will be replaced. Sixteen rats will be ordered as spares. Unused spare rats will be euthanized.

[B]Urine samples to be analyzed for uranium will be collected at selected intervals from six rats from each experimental group. These rats will not be scheduled for serial sacrifice.

[C]Serial sacrifice of rats, dosimetry, hematology, clinical chemistry, and histopathology: four rats at each of the following intervals after implantation of the 5.0 × 5.0 × 1.5 DU(Ti) fragments: 1 wk, and 1, 2, 4, 6, and 9 mo. Six rats will be sacrificed at 12 and 18 mo. The eight implant surgery controls that will be treated in parallel with the rats noted in the previous sentence will be sacrificed at 18 mo.

[d] All surviving rats will be sacrificed 2 y after implantation of the metals.

Fragments (5.0 mm × 5.0 mm × 1.1 mm) of Ta (Goodfellow Corp., Berwyn, PA) will be used as a negative control. Thorotrast® (Hyden Chemical Corp, NY) will be used as a positive carcinogenic control. The distribution, retention, and late effects of $ThO_2$ used as a radiographic contrast medium in people have been summarized by Swarm[39] and others.

The experimental design for the 2-y carcinogenesis study is summarized in Table 3. A total of 344 12-wk-old male Wistar rats (Charles River Laboratories, Wilmington, MA) will be used in this study. The Wistar strain was chosen because, in contrast to the F344, it has a relatively low incidence of nephropathology that could confound the results.[40] The Wistar rat is also larger than the F344, resulting in a larger muscle mass for implantation. In addition, survival and tumor incidence data are available.[41,42] Fifty rats per group will be required except for the sacrifice series groups (Table 3).

The rationale for the 50 rats per group is that this is the standard group size in the National Toxicology Program Statement of Work and the EPA Guidelines 40 CFR 798: 3320 - "Combined Toxicity and Oncogenicity Testing." The dose has been revised so that this study will be consistent with that used by AFRRI in a 12-mo study in rats, *i.e.*, the surface area of the 5.0 mm × 5.0 mm × 1.5 mm fragment of DU(Ti) is similar to that which resulted in weight loss in the rats with the implanted fragments.

Before implantation surgery, the DU(Ti) pellets, DU(Ti) fragments, and Ta fragments (Table 3) will be weighed, cleaned by immersion in an industrial detergent, rinsed in absolute ethyl alcohol, sterilized by immersion in a 50% nitric acid solution for 3 min, rinsed with sterile water, and placed in acetone to inhibit oxidation. This is the same procedure as used in the work at AFRRI.[37] These procedures remove the oxide formation from the surface of DU metal.[43]

Twenty-four-hour urine samples will be collected from six rats with four 5.0 mm × 5.0 mm × 1.5 mm DU(Ti) fragments and six rats with four 5.0 mm × 5.0 mm × 1.5 mm Ta implants. The urine samples will be collected daily for the first 7 d after implantation; twice per week from days 8-28; once per week from days 29-90; and once every 2 wk from day 91 through 2 y (Table 3). Rats with the implanted Ta fragments will serve as controls to determine the U background level in urine samples. Twenty-four-hour urine samples will also be collected from six rats with four implanted DU(Ti) pellets on the same schedule through 90 d. At that time, all urine samples will be analyzed for U. The results will be reviewed and a decision made regarding the need for additional urine samples from the six rats with the DU(Ti) pellets.

Rats will be entered into this study in three blocks of 96 to 102 rats each and one block of 44 rats. Those in the first three blocks will be observed for 2 y, sacrificed, and examined histologically. As noted below, urine samples will be collected from a limited number of these rats throughout this study. Forty-four rats having either 2.5 mm × 2.5 mm × 1.5 mm DU(Ti) fragments implanted or having experienced only sham implant surgery will constitute the last block. These rats will be serially sacrificed at intervals to 18 mo for dosimetry, hematology, clinical chemistry, and histopathology.

The animals on 2-y study will be observed at least twice daily and moribund or terminally ill animals euthanized. Once a week, surgical sites will be palpated for evidence of inflammation or onset of tumors. All surviving animals will be sacrificed once 90% of any one group has died or at 24 mo, whichever occurs first. A complete necropsy will be performed with examination of all organ systems, paying special attention to the implant sites and the urinary system. Histological examination will be routinely performed on the implant sites, including site neoplasms, gross lesions that are potential metastases, and the kidneys. Neoplasms at the implant sites will be characterized with light microscopy and immunohistochemistry. Ultrastructural studies of the tumor cells have implicated a pluripotential mesenchymal cell type possessing morphologic characteristics consistent with cell types of the microvasculature as the preneoplastic parent cell.[44] Thus, cell identifications will focus on endothelial cells, smooth muscle cells, and pericytes.

This protocol most closely mimics what is seen in the exposure of the Gulf War veterans to DU-containing shrapnel. This is a simple, straightforward approach; however, little work has been reported that can be used for a basis of comparison using such a route of exposure.

## III. CONCLUSIONS

We have demonstrated that the subcutaneous implant carcinogenesis system described by Brand *et al.*[16] is not an appropriate one in which to study the carcinogenesis of implanted DU(Ti) fragments in rats. An alternate method, intramuscular implantation of DU(Ti) fragments, will be used to study the carcinogenic effects of implanted DU(Ti) fragments. These studies will be finished in June 1999 and will require a no-cost extension of the contract period.

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## V. ADDENDA

### 1. Acronym and Symbol Definition

| | | |
|---|---|---|
| AFRRI | = | Armed Forces Radiobiology Research Institute |
| CPH | = | Cox proportional hazard |
| DU | = | Depleted uranium |
| DU(Ti) | = | Depleted uranium + 0.75% titanium |
| Ta | = | Tantalum |
| U | = | Uranium |

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